XLV. THE OXIDATION OF LECITHIN AND OTHER FATTY SUBSTANCES IN THE PRESENCE OF GLUTATHIONE.

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(Received January 1st, 1936.)

THE theory has frequently been advanced that the liver is intimately connected with oxidative processes involving the metabolism of fat in the body. The well-known desaturation theory, according to which a desaturation of fatty acids occurs in the liver giving a more oxidisable form of fat which is then passed on to the tissues, has been linked particularly with the work of Leathes and Meyer-Wedell [1909] and of Hartley [1909]. The validity of the desaturation theory and of the findings on which it rests have been questioned by Aylward *et al.* [1935] and by Turner [1930].

The possible relation of lecithin to fat metabolism has been made the subject of numerous investigations particularly by Bloor [1916], Artom [1931] and Sinclair [1932]. Jost [1931] showed that in perfusion experiments phosphatide was taken up by the liver, with the formation of ketone and an increase in the oxygen uptake of the organ, and concluded that the breakdown of fatty acids occurs through the intermediary of phosphatide.

In addition to its high lecithin content, the liver is also known to contain a large amount of the sulphhydryl compound glutathione, which is thought to play an active part in the oxidation mechanisms in animal tissues, and which also has been found to be capable of bringing about the oxidation of fat [Hopkins, 1921; 1925]. A similar increase in the oxygen uptake of fats with other sulphhydryl compounds was observed by Meyerhof [1923], whilst the effect of amino-acids was studied by Franke [1932] and by Page and Bülow [1935]. The latter workers extended previous investigations on the catalytic action of metallic salts and observed a marked increase in the rate of oxidation, particularly of kephalin, in the presence of iron.

The present investigation was suggested by a consideration of the fact that the liver is known to contain a readily oxidisable form of fat in the shape of lecithin and in addition a substance (glutathione) having oxidation-reduction capacities which had previously [Hopkins, 1921; 1925] been shown capable of bringing about the oxidation of fat. The experiments were devised with the purpose of determining whether the combination of fatty acid radicals in the lecithin molecule would render them more susceptible to oxidation than would be the case if they were exposed to the glutathione system either in the free state or as neutral fat.

EXPERIMENTAL.

The glutathione used in these experiments was prepared in the reduced form by the procedure of Pirie [1930]; a specimen in the oxidised condition was obtained by mild treatment with hydrogen peroxide by Schöberl's method [1931]. The phosphatides were freshly prepared according to the procedures used in a previous investigation [King, 1934]. Several specimens of purified fatty acids and glycerides were furnished by Dr A. D. Barbour of the Ontario

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Research Foundation. Our thanks are due to him, and to Dr C. S. Hanes of the same institution for the loan of a Barcroft apparatus.

Anaerobic oxidations. The experiments constituted an attempt to discover if, under any circumstances, glutathione was capable of oxidising lipid by transferring hydrogen atoms from the lipid to a reducible dye. For this purpose Thunberg tubes were employed. Hopkins's experiments with methylene blue, glutathione and lipid were repeated and his negative results confirmed.

o-Cresolindophenol, which decolorised immediately with reduced glutathione, was used in a series of experiments with lipid and oxidised glutathione. Glutathione solutions (5 mg. per ml. of buffer) and emulsions of lipid (10 mg. per ml. of buffer) at p_H 8.4 were made up fresh each day. (Triethanolamine was used as an emulsifying agent.) A solution of 1 : 2500 *o*-cresolindophenol was found to be of a suitable strength and to keep well if preserved in the dark. The test solutions for a typical experiment were set up as follows:

1 ml. emulsion (10 mg. fat) + 1 ml. buffer + 0.5 ml. *o*-cresolindophenol + 1 ml. glutathione solution (5 mg. G.S.S.G.¹).

The emulsion consisted in (1) of lecithin, in (2) of fatty substance other than lecithin and was replaced by water in a control experiment.

Lecithin induced the most rapid colour change. After lecithin came lysolecithin, kephalin and hydrolecithin followed by the fatty acids in the order of their iodine numbers. The triglycerides, even those with a comparatively high iodine number, decolorised the dye practically no faster than did glutathione alone (Table I).

Table I. *Decoloration of o-cresolindophenol by fatty substances with glutathione.*

Compound	Iodine number	Time for total decoloration min.
I. Liver lecithin	77	110
Egg lecithin	48	120
Kephalin	84	130
II. Lysolecithin	0	136
Hydrolecithin	0	188
Synthetic lecithin	0	264
III. Linolic acid	139	190
Oleic acid	81	256
IV. Linseed oil	120	216
Olive oil	81	256
V. Palmitic acid	0	280-320
Stearic acid	0	280-320
Tripalmitin	0	280-320
Distearin	0	280-320
Triolein	58	280-320
G.S.S.G.	—	280-320

Aerobic oxidations. The influence of glutathione upon the aerobic oxidation of lipoids was investigated by means of the Barcroft apparatus. Duplicate experiments were run as follows:

	(1)		(2)		(3)		(4)	
	Left cup	Right cup	Left cup	Right cup	Left cup	Right cup	Left cup	Right cup
2 ml. lecithin (20 mg.) in phthalate buffer	+	+	+	+				
2 ml. fatty acid (20 mg.) in phthalate buffer					+	+	+	+
1 ml. G.S.H. ² (8 mg.) in phthalate buffer		+		+		+		+
1 ml. phthalate buffer	+		+		+		+	

¹ G.S.S.G. = oxidised glutathione.

² G.S.H. = reduced glutathione.

The p_H in all experiments was 3.5¹ and the temperature 38°. Phthalate buffer was used throughout; a preliminary experiment with veronal buffer showed an inhibitory effect upon oxidation.

Several experiments were carried out in this way to test the comparative rates of oxygen uptake by lecithin, unsaturated fatty acids and a triglyceride. The results (Table II) indicated that oxygen was absorbed much faster by the

Table II. *Oxygen uptake of egg lecithin, fatty acids and triglyceride.*

	Iodine No.	O ₂ uptake (μl.)	
		220 min.	340 min.
20 mg. egg lecithin	65	340	440
20 mg. linolic acid	139	252	354
20 mg. oleic acid	80	48	62
20 mg. triolein	58	26	40

lecithin emulsions than by those of the fatty acids or of the neutral fat (triglyceride). The inference would seem justifiable that fat in the form of lecithin is more readily oxidisable under the action of glutathione than are other forms of fat.

A promising means of establishing whether or not the chemical combination of a fatty acid in the lecithin molecule leads to a more ready susceptibility to oxidation in the glutathione system was to compare the actual fatty acids, obtained from lecithin on hydrolysis, with the parent lecithin. Barium hydroxide hydrolyses were carried out on several different lecithins and the products of hydrolysis isolated according to the procedure of Channon and Chibnall [1927]. The results of a typical set of experiments are given in Fig. 1. The oxygen uptake of lecithin is roughly about four times that which would be expected from its iodine number in relation to the iodine numbers of its constituent fatty acids and their oxygen uptake.

Experiments upon brain lecithin and brain kephalin were also performed and the results are given in Fig. 2. In spite of the fact that the brain lecithin had a slightly lower iodine number than kephalin, its oxygen uptake was almost twice as great. As in the case of egg lecithin, the hydrolysis products of brain lecithin were found to have a much lower oxygen uptake than that of the parent lecithin in spite of their higher iodine number.

Lysolecithin and hydrolecithin displayed a very slow rate of oxygen uptake. When mixed with lysolecithin, unsaturated fatty acids absorbed oxygen somewhat more slowly than when exposed alone. This was probably due to the fact that there was less actual fatty acid present in the mixture, a proportion of the 20 mg. (the quantity of fatty substance invariably used) being practically non-oxygen-absorbing. The slow rate of aerobic oxidation of lysolecithin is in contrast to the fairly rapid rate of decolorisation of *o*-cresolindophenol in the anaerobic oxidations (Table I).

As was to be expected, no carbon dioxide was given off during the oxidations of the fatty substances.

It is possible to calculate the degree to which the fatty substances used in these experiments were oxidised, using as a criterion their iodine numbers on the basis of each atom of iodine being the equivalent of half an atom of oxygen.

¹ Hopkins emphasised the importance of the hydrogen ion concentration of his systems, pointing out that tissue oxidation took place most rapidly at p_H 7.4 and lipid oxidation at about p_H 3.5.

Fig. 1.

Fig. 2.

Fig. 1. Oxygen uptake of egg lecithin and its hydrolysis products. *A*, egg lecithin (Iodine No. 65); *B*, unsaturated fatty acids from hydrolysis (Iodine No. 101); *C*, Purified mixed fatty acids from hydrolysis (Iodine No. 85); *D*, constituents of lecithin mixed in molecular proportions; *E*, crude fatty acids from hydrolysis (Iodine No. 78); *F*, saturated fatty acids from hydrolysis.

Fig. 2. Oxygen uptake of brain lecithin, its hydrolysis products and brain kephalin. *A*, brain lecithin (Iodine No. 51); *B*, brain kephalin (Iodine No. 54); *C*, mixed fatty acids from brain lecithin (Iodine No. 65).

Table III. *Oxygen uptake of fatty substances with glutathione in relation to their iodine numbers.*

Compound	Iodine No.	Oxygen uptake in 340 min. μ l.	% of total theoretical oxygen uptake*
Egg lecithin	65.5	440	76.2
Crude fatty acids from hydrolysis of egg lecithin	78	52	7.6
Purified fatty acids from hydrolysis of egg lecithin	85.4	92	12.2
Unsaturated fatty acids from hydrolysis of egg lecithin	101	200	22.5
Constituents of egg lecithin mixed in molecular proportions	—	54	12.2
Brain lecithin	51	216	48.0
Purified fatty acids from hydrolysis of brain lecithin	65	36	6.3
Brain kephalin	54	112	23.5
Linolic acid	139	354	28.9
Triolein	58	40	7.8
Oleic acid	93.4	62	7.5

* Calculation performed as follows: *e.g.* linolic acid, iodine number 139. 100 mg. linolic acid $\equiv$ 139 mg. iodine $\equiv$ 8.76 mg. oxygen = 6.12 ml. Actual oxygen uptake of 100 mg. linolic acid = $\frac{0.354}{20} \times 100 = 1.77$ ml.; % of theoretical oxygen uptake = $\frac{1.77 \times 100}{6.12} = 28.9$.

Table III shows that lecithin absorbed a very considerable fraction of its theoretical oxygen equivalent; contrasted with this are the much smaller figures for the unsaturated acids and the other forms of fat.

DISCUSSION.

Careful microscopic examination of the emulsions used in these experiments revealed the fact that at p_H 3.5, those of lecithin and free fatty acids contained particles of approximately the same size. (It is also of interest to note that Hopkins [1925] stated that the size of particles had no influence on the rate of oxygen uptake in the Barcroft apparatus.) It would therefore appear that the extra oxygen uptake of lecithin over that of its constituent fatty acids is a chemical phenomenon and that the configuration of the lecithin molecule renders it peculiarly susceptible to oxidation in the glutathione system. The mere presence of phosphate and choline in an emulsion of fatty acid induces no further oxygen uptake than would be expected from the oxidation of fatty acid alone. That the presence of phosphate in the lecithin molecule is not solely responsible for its ease of oxidation, but that choline also plays a part, may be inferred from the fact that lecithin (which contains choline) is more rapidly oxidisable than kephalin, which contains phosphorus but has amino-ethyl alcohol instead of choline for its base.

Page and Bülow [1935] found kephalin to be more rapidly oxidised than lecithin when iron or copper was present as catalyst (whereas in the present experiments with glutathione the reverse was the case). Tests for iron in the mixtures used revealed the presence of about 6γ Fe [Page and Bülow used 0.3 mg.], most of it being in the glutathione. The addition of small amounts of extra iron was without effect. Varying amounts of cyanide up to 1 mg. failed to alter the oxygen uptake with any of the lipid materials tried, although larger amounts (5–20 mg.) caused a graded inhibition with almost complete suppression of the oxygen consumption when more than 20 mg. of cyanide were present. 1 mg. of cyanide is more than sufficient to suppress any activity which might be due to the 6γ of iron. It is therefore improbable that iron acting catalytically affected the rate of oxygen uptake or that it was concerned in the glutathione catalysis of the aerobic oxidation of the fatty substances. Meldrum and Dixon [1930] detected no difference between "impure" and crystalline glutathione in the oxygen uptake induced with linolic acid, although the crystalline substance required the presence of iron and an "activator" to bring about the oxidation of protein.

In aerobic fat oxidation by glutathione the sulphydryl-disulphide relation is apparently not involved [Allot, 1923; Meldrum and Dixon, 1930]. The 8 mg. of reduced glutathione used in the experiments should require $146 \mu l.$ of oxygen for conversion into the disulphide, but in blank experiments no evidence of this transformation could be obtained, and in the fat oxidations the glutathione was always present in the reduced form at the termination of the experiment. Substitution of oxidised for reduced glutathione failed to elicit an oxygen uptake in excess of that in the controls. In all experiments with lecithin and in many of those with unsaturated fatty acids the oxygen absorbed was greatly in excess of the oxygen equivalent of the glutathione present, which would appear to have acted in a catalytic fashion to influence the rate of oxygen uptake.

SUMMARY.

1. A comparison of the rates of oxidation in the presence of glutathione, of lecithin, cephalin, neutral fats and fatty acids, including those obtained from lecithin on hydrolysis, has been made.
2. Under anaerobic conditions at a slightly alkaline reaction, lecithin appears to be much more rapidly oxidised by means of oxidised glutathione than is neutral fat or fatty acid.
3. At an acid reaction the oxygen uptakes of lecithin and reduced glutathione are greater than that of the neutral fat or fatty acids tested and also greater than that of the fatty acids obtained from lecithin on hydrolysis.
4. The oxygen uptake of lecithin is about four times that which would be expected from a consideration of its iodine number in relation to the iodine number of its constituent fatty acids and their oxygen uptakes.
5. Calculated on the basis of iodine numbers the oxygen uptake of lecithin is a much higher percentage of the theoretical than is the case with unsaturated fatty acids.

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